Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Lipid content assessment of feline oocytes in vitro maturation (IVM)

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Resumo

The domestic cat has been considered an important experimental model for the application of reproductive biotechnologies on aiming the conservation of endangered felids. The intracellular lipid content and composition have an important role in oocyte viability during cryopreservation. Some studies have already demonstrated an increase in lipid content within extended IVM time duration in some species, such as bovine and swine. The aim of this study was to investigate the time course of lipid accumulation during IVM in feline oocytes. For reaching that, oocytes were recovered from feline ovaries obtained in elective surgeries and, after selection, transferred to IVM (TCM-199 hepes, 4 mg/mL BSA, 0.2 mM piruvate, 50 ug/mL penicillin-streptomycin, 0.5 ug/mL FSH, and 1 ug/mL estradiol, at 37 ℃, in maximum humidity) for 24, 28 and 32 h. For lipid content evaluation, oocytes from the three experimental groups (G24, G28, and G32), plus immature oocytes (GI), were fixed in 4% paraformaldehyde solution for 40 min and stored in phosphate-buffered saline at 4 ℃. Then, all structures were stained with Oil Red O solution (Sigma Chemical Co.). Oocytes were washed in a 50% ethanol solution for 2 min, stained for 15 min in Oil Red O solution and washed three times, for five min each, in 50% ethanol solution. After, they were kept for five min in distilled water before being evaluated. Images of each structure were captured using phase-contrast microscope and evaluated for the stained area fraction using Image J software (NIH Image, Bethesda, MD, USA). The lipid content results were submitted to ANOVA. The Tukey test was used for comparison among groups. A total of 37 oocytes were used (GI: n=7/ G24: n=11/ G28: n=11/ G32: n=8), which were obtained in three replicates. The oocytes from GI had a lower ($P<0.05$) lipid content compared to those of G28 and G32 (46.4%a x 72.3%b x 74.7%b, respectively), and similar to 24 h (67.7%a), although the lipid content had increased about 50% in the latter. Considering specifically the three IVM timepoints (24 h, 28 h, 32 h), no difference ($P>0.05$) was observed in lipid content. It was concluded that a significant increase in lipid content can be observed in oocytes after 28 h of IVM, suggesting that like in other species, IVM also causes lipid accumulation in feline oocytes.

Keywords: domestic cat, IVM, lipids, oocyte.

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Embriology, developmental biology and physiology of reproduction

PRE-FERTILIZATION APPROACH USING α-L-FUCOSIDASE MODULATES ZONA PELLUCIDA HARDENING DURING BOVINE IN VITRO EMBRYO PRODUCTION

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Resumo

Polyspermy incidence during in vitro fertilization is still an important limitation for bovine in vitro embryo production technique advance, resulting in reduced embryo development, early embryonic death, or miscarriage. The glycoprotein α-L-fucosidase (FUCA), a glycosidase found in mammalian oviductal fluid, has been described to be involved in the hydrolytic degradation of fucose as well as to participate in sperm-oocyte binding through interactions with complementary glycans on the surface of the zona pellucida (ZP), acting in the control of polyspermy. Therefore, our objective was to investigate the effect of the addition of FUCA during in vitro pre-fertilization on the modulation of the ZP hardening, embryonic development, and quality of bovine blastocysts produced in vitro. For that, after in vitro maturation, bovine cumulus-oocytes complexes (COCs, at least 100 COCs/treatment/experimental analysis) were incubated for one hour with FUCA at four different concentrations (0; 0.0625; 0.125; and 0.25 IU/mL). Subsequently, the treated COCs were fertilized and the probable zygotes intended for evaluation of embryonic development in vitro. During the experimental analyses we evaluated the embryo production rate, ZP digestion time, and monospermic fertilization rate. Embryonic quality was evaluated by analysis of marker genes of pluripotency, differentiation, implantation, and embryonic development. The effect of FUCA addition was evaluated by ANOVA and, when present, the means were compared by Tukey test. The differences were considered significant when $P<0.05$. There was no difference ($P>0.05$) in the blastocyst rate when comparing the groups 0; 0.0625; 0.125; and 0.25 IU/mL (46,1±17,3; 48,2±7,8; 34,9±6,9; 33,4±10,9%, respectively), however, the number of blastocysts that hatched after treatment with FUCA at the concentration of 0.0625 IU/mL was more than twice (n=15, 21,4±4%) as many blastocysts hatched as the control group (n=6, 10,4±10). Additionally, the addition of 0.0625 IU/mL of FUCA during pre-fertilization of COCs increased ($P<0.05$) the ZP digestion time (344±102 seconds) compared to 0; 0.125; and 0.25 groups (279±68; 300±104, 289±93 seconds, respectively), suggesting an important role of FUCA in the control of the ZP hardening. When analyzing the sperm penetration assay, we did not observe any difference between treatments, but the addition of 0.0625 IU/mL of FUCA during pre-fertilization demonstrated a biological tendency to increase monospermic fertilization ($P=0.10$). When we prospected the quality of blastocysts through the transcription profile, treatment with FUCA did not modulate the abundance of OCT4, PLAC8, CDX2, SOD2, and VEGF mRNA ($P>0.05$). We believe that FUCA does not alter the production of bovine blastocysts in vitro, however, it positively modulates the ZP hardening and seems to improve the performance of monospermic fertilization.

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Embriology, developmental biology and physiology of reproduction

Placental development and thermoregulation in pregnant sheep in the silvopastoral system during the summer

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Resumo

Animals subjected to high temperatures undergo behavioral, physiological, and reproductive changes. Thus, this study aimed to analyze different reproductive aspects and physiological changes in ewes subjected to heat stress during pregnancy. This experiment was carried out at the Sheep Research Unit at UTFPR, in Dois Vizinhos, PR. Pregnant crossbred Dorper x Santa Inês ewes were divided into two groups: Silvipastoril System (SPS; n=12) and Open Pasture (OP; n=12) on the day 43,4±9,17 of pregnancy. Physiological variables and environmental data were registered every two weeks for six time points from this moment forward. The ewes were kept in a suspended pen from day 123,4±9,17 of pregnancy until parturition. Placentas and lambs were weighed at birth, and the placentas were photographed for biometrical analysis. Lambs were also weighed at ten days of age. Both systems were stressful for the sheep when all microclimatic variables were taken into account, but the SPS had lower Air Temperature than OP (OP=26.9±0.41°C, SPS=26.0±0.38°C; p=0.0288; T Student test). Moreover, the radiant thermal load of the two groups presented a difference of 34Wm-2 (p=0.0288), and the Temperature of the Grass was also different (PS=25.6±0.44°C, SSP=23.4±0.37°C; p=0.0043). During the study, no system effect was observed on the mobilization of white blood cells (p=0.4777), nor was there any effect of time or interaction between variables (p=0.8109 and p=0.4150). No differences were observed in quantifying circulating monocytes between the groups (p>0.05). Neutrophils were only affected by time (p<0.0001). In the SPS group, a difference was observed between timepoints 4 and 1, 5 and 1, 6 and 1 (p=0.0174; p=0.0093; p=0.0065, respectively), between 4 and 2, 5 and 2, 6 and 2 (p=0.0096; p=0.0050; p= 0.0035, respectively). While in the OP group, differences were observed between timepoints 5 and 1 and 6 and 1 (p=0.0328; p=0.0204, respectively). Respiratory and Heart Rates of the animals exposed to the sun were higher than that of the sheep that remained in the shade (p<0.001). Regarding the duration of pregnancy, there was no effect of treatment (p=0.4987). Interestingly, both systems had higher numbers of female lambs (PS: male 40%, female 60%; SSP: male 38%, female 61.54%). Only an effect of the type of pregnancy (single vs. twin) was detected on the bodyweight of lambs at ten days (p=0.0273), which was not observed at birth (p=0.9455). Regarding placental biometry, twin pregnancies had a greater membrane area (p=0.0223), but no differences were observed in placenta weight (p=0.1522) and the number of cotyledons (p=0.5457). Therefore, it can be concluded that the type of Sheep Rearing System affects the thermal comfort of pregnant ewes, and the SPS can offer more amenable microclimate conditions resulting in more comfort during pregnancy.

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Embriology, developmental biology and physiology of reproduction

Developmental rates and quality of blastocysts generated by zinc chelation and intracytoplasmic calcium rise of porcine eggs.

Juan Pedro Cabeza [1], Juan Camera [2], Olinda Briski [1,3], Daniel Salamone [1,3], Andres Gambini [1,2,3]

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Resumo

Immediately upon the sperm-egg fusion, orchestrated rises of intracytoplasmic calcium (Ca) are followed by the release of zinc (Zn) out to the perivitelline space (zinc sparks). Both elements play an essential role in triggering oocyte activation and embryo development. Our work aimed to determine the optimal condition for porcine egg activation using the Zn chelator 1,10-phenanthroline monohydrate (PHEN, Sigma-Aldrich, CABA,Argentina, Product Number: P9375) and to compare embryo developmental rates and quality of eggs activated with the Ca2+ ionopohre -ionomycin- (IONO), known to increase intracytoplasmic levels of Ca, and consequently induction of the Zn sparks. First, we compared PZM and TALP-H media for zinc chelation at a previously published concentration for pig (0.5mM for 1h; Uh et al., Theriogenology, 125:259-267, 2019; Experiment 1). Afterwards, we determined the optimal concentrations and exposure time for PHEN treatment (Experiment 2). Finally, we compared the effects of Zn chelation before and after ionomycin induction of an intracytoplasmic Ca rise (Experiment 3). Blastocyst quality was determined by immunofluorescence (IF) of SOX2, OCT4 and CDX2. Oocyte collection and IVM were performed as reported (Buemo et. al., PLoS ONE 11(2): e0146390, 2016). Eggs were activated using PHEN or IONO (5mM for 4m in TALP-H). After treatment, zygotes from all experimental groups were incubated for 3h in 1.9mM of 6-Dimethylaminopurine. Embryos were cultured in microdrops of PZM media. Day 7 blastocysts were fixed and subjected to IF analysis using SOX2, OCT4 and CDX2 antibodies (Gambini et al., PLOS ONE 15(9):e0238948, 2020). PHEN in TALP-H resulted in higher blastocyst rates than in PZM (IONO, n=62, 17,74%; PHEN-PZM, n=92, 3,26%; PHEN-TALP-H, n=93, 15,05%) and it was used for experiments 2 and 3. Embryo developmental rates with PHEN 1mM for 30m in TALP-H was higher (n= 85, 44,71%) than IONO (n=97, 20,62%) or other PHEN conditions (PHEN 0.5mM for 1h n=146, 18,49%; PHEN 1mM for 1h n=63, 14,29%). IONO and PHEN blastocysts had similar total cell number (mean ± SEM; n=7, 41.71 ± 3.12 and n=8, 34.13 ± 6.88, respectively) and no significant differences were found in the number of nuclei or expression pattern of the studied markers. Interestingly, Zn chelation after (n=103, 31,07%) or before (n=76, 21,05%) a Ca oscillation impaired blastocyst rate compared to Zn chelation only (n=76, 46,05%) but not with IONO (n=79, 27,85%). In conclusion, we have established new optimal conditions for oocyte activation using PHEN without affecting blastocyst cell number nor the expression of relevant transcription factors, suggesting that Ca oscillations are not essential for their normal in vitro expression in parthenogenetic embryos in pigs. Moreover, the artificial manipulation of both Zn and Ca, in any order, negatively affects embryo development at the concentration tested, possibly for interrupting the orchestrated mechanism needed proper oocyte activation.

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Embriology, developmental biology and physiology of reproduction

Antral follicular count in Bos indicus (Nellore) and Bos taurus (Caracu) prepubertal heifers

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Resumo

Antral follicular count (AFC) is the determination of the ovarian antral follicles number (≥ 2 mm), which while is highly variable in the cattle, has high repeatability in the same animal. However, there are still scarce and conflicting reports regarding this characteristic in prepubertal heifers and its repeatability at different follicular wave moments. Thus, the objective of this study was to evaluate the effects of the follicular wave moment (emergence and dominance) and of the breed (Nellore and Caracu) on AFC, and determine its repeatability and variability in prepubertal heifers. Nellore (Bos indicus; n = 30) and Caracu (Bos taurus; n = 28) heifers were previously selected by B-Mode ovarian ultrasound evaluations that confirmed the prepubertal anestrus condition (absence of corpora lutea). The females' mean age and weight were 468 ± 25 days and 236.7 ± 26.7 kg for Nellore and 468 ± 20 days and 282.1 ± 25.9 kg for Caracu. A sequence of eleven ultrasound evaluations was conducted every 48 hours (Day 0 – random day of the follicular wave, until Day 20) to quantify the antral follicles. The dominant follicle (DF) was also identified and had its diameter measured. Data obtained were compared according to the follicular wave moments, which were defined by the absence or presence of a DF, considered as the largest ovarian follicle visualized, with a diameter ≥ 6.2 mm for Nellore and ≥ 8.5 mm for Caracu breed. Statistical analysis included fixed effects of evaluation days, breeds and their statistical interaction, being performed by the MIXED, GENMOD, CORR and GLM procedures of the SAS statistical program ($P < 0.05$). The repeatability coefficient was obtained as the ratio of the between-animal variance to the total variance (between-animal + within-animal). At least 80% of Nellore and 46% of Caracu heifers were in the follicular dominance phase on each study day. The DF diameter was greater in Caracu than in Nellore heifers (9.9 ± 0.9 mm vs. 8.8 ± 1.4 mm; $P < 0.0001$) and varied throughout the days only in the zebu ones. The AFC was greater in the Nellore than in the Caracu breed (30.2 ± 14.8 vs. 15.7 ± 8.2; $P < 0.0001$). In Nellore heifers, a gradual increase in AFC was observed from D0 to D6, followed by a decrease to D10, remaining until D20. In Caracu heifers, the greatest AFC increase was observed from D10 to D12. The follicular wave moment also influenced the AFC, which was greater in both breeds in the dominance than in the emergence phase of the follicular wave (29.7 ± 1.5 vs. 27.4 ± 1.8 for Nellore and 16.1 ± 1.6 vs 14.9 ± 1.7 for Caracu, respectively; $P < 0.05$). The AFC repeatability was 0.76 and 0.74, even it varied from one to 89 and three to 48 follicles, for Nellore and Caracu heifers, respectively ($P \leq 0.0002$). In conclusion, although the breed and the follicular wave moment affect AFC, it has high repeatability and a single measure throughout prepuberty is sufficient to characterize the females, as it is intrinsic to the individual.

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Embriology, developmental biology and physiology of reproduction

Effect of cGMP synthesis stimulation on the lipid content of bovine oocytes and cumulus cells during in vitro maturation

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Resumo

Lipids are important energy source for cells, but excessive accumulation renders them more likely to undergo oxidative stress. Studies have indicated that cGMP pathway may be involved in the lipid metabolism of bovine COCs during IVM. The aim of this study was to compare the effect of different stimulators of cGMP synthesis (NPPB=natriuretic peptide type B and PTPF=protoporphyrin IX, activators of membrane and soluble guanylyl cyclase, respectively) on the lipid content in bovine oocytes (OO) and cumulus cells (CC) after 9 and 24h IVM. COCs (20-25/group/replicate) were matured in TCM199 (0.2 mM sodium pyruvate, 10 µg/ml gentamicin, 0.5 µg/ml FSH, 10% FCS) with NPPB (10-7 M, Schefer et al, Anim Reprod, v.15, p.442, 2018) or PTPF (10-5 M, Schwarz et al, Theriogenology, v.81, p.556-564, 2014) or without stimulators (control). In Experiment 1, lipids were assessed in denuded OO (9 and 24h IVM), stained with Nile Red (1 µg/ml for 15 min), imaged by epifluorescence microscopy and fluorescence intensity (FI) was measured by ImageJ. In Experiment 2, lipids were assessed in CC (9 and 24h IVM), by staining COCs with BODIPY 493/503 (20 µg/ml for 1 h), which were imaged by confocal microscopy. Four random 1µm2 CC areas were selected from each image and lipid area determined in images using ImageJ nucleus counter (lipid area/ total cells area). Data (five replicates/group) were tested for normality of results and homogeneity of variance, then subjected to statistical analysis by ANOVA followed by Tukey test (GraphPad Prism software) at 5% significance level. In Experiment 1 analyzing OO at 9h IVM, lipid FI for NPPB (3.09±0.04) was lower than control (3.28±0.05, $P<0.05$) and PTPF (3.80±0.05, $P<0.05$), which was in turn higher than control ($P<0.05$). At 24h, NPPB (3.01±0.07) remained lower ($P<0.05$) than control (3.31±0.06) and PTPF (3.34±0.07), which did not differ from the control ($P>0.05$). In Experiment 2 analyzing CC at 9h IVM, no difference in lipid area/µm2 was observed (0.018± 0.03 to 0.039±0.018, $P>0.05$), but at 24h, NPPB (0.008±0.001) was lower ($P<0.05$) than control (0.019±0.002) and PTPF (0.021±0.006), which was similar to control ($P>0.05$). In conclusion, stimulators of different cGMP synthesis enzymes show distinct effects on lipid amounts in bovine COCs and dependent on IVM time; NPPB was more effective to reduce lipids in both OO and CC.

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Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Development and quality of embryos generated by zinc chelation of bovine eggs

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Resumo

Oocyte activation is considered one of the most crucial steps for successful embryo development. Although naturally triggered by sperm, oocyte activation can be achieved by artificial means to improve the development of fertilized eggs or to produce nuclear transfer embryos. After sperm-egg fusion, intracytoplasmic rises of calcium (Ca) induce the release of zinc (Zn) out of the egg (Zn sparks). Both phenomenons are known to play an essential role in the oocyte activation process. Our work aimed to determine the optimal condition for inducing activation of bovine eggs using the novel Zn chelator 1,10-phenanthroline (PHEN, Sigma P9375) and to compare the parthenogenetic developmental rates and embryo quality with IVF and -Ionomycin- induced (IONO) embryos. In Experiment 1, we compared SOF and TALP-H media for Zn chelation at an established condition (0.5mM for 1h; Uh et al., Theriogenology, 125:259-267, 2019). In Experiment 2, we compared incubation conditions to the Zn chelator for optimising the activation protocol. Embryo quality was assessed by immunofluorescence (IF) of SOX2, SOX17 and CDX2. Oocyte collection, IVM and IVF procedures were performed as reported (Ynsaurralde et al., Theriogenology, 148:140-148, 2020). Eggs were activated using PHEN or 5mM of IONO for 4m in TALP-H. After treatment, zygotes were incubated 3h in 1.9mM of 6-Dimethylaminopurine and cultured in SOF media. Fisher's exact test was performed for statistical analysis. Day 7 blastocysts were fixed and subjected to IF using SOX2, SOX17 and CDX2 antibodies followed by statistical analysis as reported by Gambini et al., PLOS ONE 15(9):e0238948, 2020. In Experiment 1, PHEN-TALP-H resulted in higher cleavage rates compared to PHEN-SOF and was used for experiment 2 (IONO, n=80, 95.00%; PHEN-SOF, n=73, 61.64%; PHEN-TALP-H, n=93, 76.34%). PHEN blastocyst rates were significantly lower compared to the control (IONO, 76,25%; PHEN-SOF, 3,26%; PHEN-TALP-H, 15,05%). In Experiment 2, PHEN developmental rates were lower than embryos with artificial (IONO) or sperm-induced (IVF) activation (IVF, n=101, 41.58%; IONO, n=83 50.60%; PHEN 0.5mM for 30m, n=73 20.55%; PHEN 0.5mM for 1h, n=82, 28.04%; PHEN 1mM for 30m, n=72, 27.78%; PHEN 1mM for 1h, n=72, 19.44). Blastocyst produced with PHEN 0.5mM for 1h showed a significantly less total cell number compared to IVF (mean±SEM, IVF 116.5±7.56; IONO, 91.00±7.30; PHEN 85.19±5.16). Moreover, PHEN blastocysts showed a higher number of SOX2+ cells (52.70±4.5) than IVF (29.80±4.5), but not with IONO (43.03±6.68). Interestingly, more than 40% of the PHEN embryos showed a scattered pattern of SOX2 expression compared with less than 15% in IONO and IVF groups. Our observations suggest that even though blastocyst development can be achieved in vitro using a Zn chelator in bovine bypassing Ca oscillations, developmental rates and blastocyst quality are compromised compared to embryos generated with artificial or sperm-induced calcium oscillations.

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Embriology, developmental biology and physiology of reproduction

In vitro development of mule ICSI embryos

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Resumo

Horses, donkeys, and zebras belong to the sole genus Equus of the family Equidae. The existence hybrids of female horses (mares) with male donkey (jacks), known as mules, is documented back to at least 3000 years ago. Domestic horses, donkeys and their hybrids are used worldwide as work or pack animals, for leisure or other equestrian activities, for meat/milk production and as companions animals. The rapid development of assisted technologies in the last 10 years such as the ovum pick up (OPU), the intracytoplasmic sperm injection (ICSI), and the somatic cell nuclear transfer in the horse industry has allowed its recent application to other members of the genus. Up to date, only hybrid blastocysts between horse and zebra were reported and, to our knowledge, no in vitro ICSI blastocysts from donkeys or their related hybrids have been published. This work aimed to compare the developmental competence after ICSI of mare eggs injected with horse (horse) or donkey (mule) sperm cells. Cryopreserved semen from one donkey and two stallions we used for this study. Cumulus oocyte complexes (COCs) were transvaginally aspirated from Percheron mares of 5 to 9 years old. Recovered COC's were placed in equine holding media and transported to the laboratory (12-24 hours) at 20 oC. In vitro maturation, oocyte denudation and ICSI was performed as described by Gambini et al., PLoS One. 11;15(9):e0238948, 2020. Embryo culture was performed in commercial media IVF bioscience (Eq-IVC-1 for 3 days followed by eq-IVC-2 supplemented with 10% of FBS for 10 days). Blastocysts were identified daily from day 7 up to day 10 and vitrified for future embryo transfer. Fisher's exact test was performed for statistical analysis. No significant differences were found on cleavage rates (n=42, 59,54%; n=82, 65,85%), blastocyst rate at day 8 (7,14% vs 4,8%) or blastocyst rate at day 10 (14,28% vs. 19,51%), between horse and mule embryos respectively. Blastocyst rates considering cleaved embryos was 36% for horse and 37% for mule. Our results indicate that donkey sperm can induce horse oocyte activation after ICSI without compromising the in vitro developmental blastocyst rates. To our knowledge, this is the first report of a successful in vitro blastocyst production derived from OPU eggs recovered from live mares injected with cryopreserved donkey sperm and it encourages the study of in vitro equine hybrids and the combination of genetic for production and conservation of high valuable animals.

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Embriology, developmental biology and physiology of reproduction

Establishment of a tridimensional (3D) organoids culture using bovine endometrial glandular epithelial cells

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Resumo

In ruminants, the pregnancy begins with the migration of the blastocyst into the uterus. At this stage, the uterus supports the early embryo development up to the embryo implantation. The endometrium is composed by different cell types, including epithelial luminal, epithelial glandular and stromal cells. This cells play an important role in the uterine environment, improving receptivity to the embryo, implantation, and supporting conceptus elongation. Therefore, the main objective of this project is to establish the culture of tridimensional (3D) cell-derived bovine endometrial glandular epithelial cells to generate a model to study in vitro maternal embryo communication. The main hypothesis of the project is that the culture of organoids from glandular epithelial cells is feasible in cattle and may serve to study in vitro maternal embryonic communication. For the isolation of glandular epithelial cells, non-pregnant uteruses were selected in early luteal phase, based on the CL. The tissue dissection was performed in the Ipsi lateral horn and in the intercaruncular region. The tissue was incubated in 5mL of digestion solution (1mg/mL of collagenase) for 1 hour at 37°C, subsequently filtered through a 100 and 40 µm mesh and washed with 10% FBS in PBS Ca2++ and Mg2++ free and centrifuged at 600g/10min. The cellular precipitate was resuspended in 1mL of DMEM-F12 with 10% FBS and antibiotics (50µg/mL streptomycin and 50IU/mL penicillin), then they were seeded at 1 x 105 cells/mL in 75 cm2 culture flasks. After purifying the cell line, 3rd passage, the epithelial cells were cultured in 96-well plates with 5.000 cells/well in Matrigel drop for formation of 3D culture of epithelial cells. After 48 hours of culture, the cells were scraped to detach from the plate and went through two stes of centrifugations at 600g/10min, and thus replated again in 96-well plates with 5,000 cells/well. Three concentrations of Matrigel (5mg; 2.5 mg; 1.25mg) were tested. It was possible to observe the emergence of epithelial organoids after 96 hours, only at the 2.5mg concentration. Organoids showed an irregular shape at the beginning (96 hours) with a multicellular characteristic, and average area of 544.9±161 mm² and 81.4±13.9 mm of circumference. After 24 hours, the organoids became spherical, increased the area at greater diameter to 571.2±136.8mm with a lumen that was denser and frequently showed apparent cilia movement. The technique is still under development, as the project will contribute to the development of biobanks of bovine uterine organoids, to study the maternal embryonic communication and uterine receptivity within animal reproduction.

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CAPES.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Physiological behavior, biochemical profile, and oocyte quality of Nellore females under different climatic conditions

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Resumo

Climatic conditions can act as a limiting factor for bovine productivity, and this is even more significant in Brazil, which has most of the herd in the intertropical zone. The Nelore breed is known for its adaptation to high temperatures, however, there is still a lack of studies that demonstrate whether and how much climate change can interfere with the reproductive efficiency of females of this breed. Thus, the objective was to evaluate the physiological parameters, oocyte quality, and serum lipid and nitric oxide (NO) profile of females kept on pasture during wet and dry periods in Mato Grosso do Sul. Ten females with a mean age of 36 months and a mean weight of 436±63 kg was used and were kept in the same paddocks for 79 days in each experimental period. An evaluation of the availability and bromatological analysis of the pasture was carried out by the square method with two collections in each period every 30 days. Animals were weighed and the body condition score (BSC) was evaluated on random days, and the physiological parameters were evaluated (heart rate-HR, respiratory rate-RR, rectal temperature-RT and surface temperature by thermographic image of the vulvar region, ocular orbital, and lacrimal point), oocyte quality, levels of total cholesterol, HDL, VLDL, triglycerides (TG) and serum NO. In addition, temperature variations throughout the day were evaluated daily. All females were submitted to two Ovum Pick Up sessions per experimental period, and the cumulus-oocyte complexes were classified according to cytoplasm homogeneity and cumulus cells conformation around the oocyte. Statistical analysis was performed by PROC GLIMIX (SAS® University). For each experimental group, the analysis was performed considering the fixed effects of treatment on oocyte quality rate, biochemical parameters, free radicals, and physiological parameters. The wet season presented a temperature variation between 20 and 35°C and an average relative humidity (RH) of 75%, while in the dry season the temperature varied between 14 and 38°C and an average RH of 62%. The bromatological composition and pasture availability did not vary between seasons, and the same happened for the BSC and weight of the animals. In the dry period, the animals showed an increase in RR ($p=0.007$), RT ($p<0.001$) and temperature of the orbital region ($p=0.05$) and a lower rate of oocyte quality ($p=0.006$), being 57.77% viable in the waters and 24.23% in the dry. NO ($p<0.001$) and TG ($p=0.05$) were lower in the dry season when daily variations in ambient temperature averaged 24°C throughout the day. It is concluded that with the climatic challenges observed in the dry period, the females needed to trigger thermoregulatory mechanisms, which culminated in an increase of orbital temperature. It is speculated that such changes are linked to the increase in NO and TG, which may be related to the reduction in oocyte quality during this period.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Impairment on development and gene expression of bovine embryos derived from oocytes exposed to genistein

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Resumo

Genistein, the main isoflavone present in soy, affects the reproductive processes due to a potent steroidogenic action. Intrauterine exposure to genistein is able to affect the reproductive system of offspring, stimulate uterine cancer and cause changes in testicular epithelium. To understand the effects of genistein on in vitro embryo production, we aimed to evaluate the effects of genistein during COCs maturation on the production and quality of bovine in vitro-produced embryos. Therefore, ovaries from a local slaughterhouse were obtained and COCs were recovered and divided into three groups: control group (no genistein addition); GEN 100 (100 µM of genistein); and GEN 500 (500 µM of genistein). Concentrations were based on previous studies and tested by a pilot study. All the experimental groups contained the same base medium with 0.1 mM dimethyl sulfoxide (DMSO). The COCs were in vitro matured for 24 hours. After maturation, we submitted COCs to in vitro fertilization for 18 hours. Further, presumptive zygotes remained to in vitro culture for seven days. To evaluate the effects of genistein on IVEP we analyze the blastocyst yield and expression of genes related to embryo quality. The genes related to embryo quality observed were OCT4 (Octamer-binding transcription factor 4), PLAC8 (Placenta associated 8), and CDX2 (Caudal type homeobox 2); normalized with PPIA (Peptidylprolyl isomerase A - housekeeping gene). We analyzed the effect of oocyte exposure to genistein using ANOVA. Means were compared by orthogonal contrast and we considered different when $P<0.05$. For in vitro embryo production, we figure out ($P<0.0001$) that 500µM of genistein decreases blastocyst yield (13.05%) compared to GEN 100 group (46.11%) and control group (46.87%). Furthermore, GEN 500 group demonstrated lower OCT4 mRNA abundance compared to control group ($P<0.05$). On the other hand, genistein did not affect CDX2 expression ($P=0.21$). Taken together, we concluded that addition of 500 µM of genistein during COCs maturation impairs in vitro embryo development and down-regulates a key gene related to inner cells mass differentiation and embryo implantation.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

In vitro model to study the maternal-embryonic communication mediated by extracellular vesicles in cattle.

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Resumo

In ruminants, the maternal recognition of pregnancy (MRP) involves the production of interferon tau (IFNT) by the embryonic trophectoderm cells in order to prevent luteolysis. Besides the well characterized IFNT signaling, the production and release of extracellular vesicles (EVs) arose as a potential mechanism of cellular communication between the mother and the embryo during MRP. The purpose of this study was to create an in vitro model to understand the EVs roles in the regulation of critical biological processes such as maternal recognition of pregnancy and to investigate other mechanisms of maternal-embryonic communication. To test this, we generated cultures of endometrial cells (epithelial and stromal origin) and trophectoderm cells from in vitro fertilized blastocysts, and isolated EVs from their culture media. Thus far, stromal and epithelial cells lines (n = 5) were isolated, grown until the 4th passage, and characterized by immunofluorescence using anti-vimentin antibody (marker of stromal cells). Small EVs of the culture medium were obtained from two sets of ultracentrifugation at 120 000×g for 70 min (Optima XE-90 Ultracentrifuge; 70 Ti rotor; Beckman Coulter, Brea, California, USA). Isolated EVs were characterized based on size and concentration of particles using Nanoparticle Tracking Analysis (NTA). As a result, only stromal cells were positive to mesenchymal vimentin as expected. EVs showed an average size of 131.92 nm and 153.46 nm, and concentration 6.64 x108 particles/mL and 8.15 x108 particles/mL, for epithelial and stromal cells, respectively. There was no significant difference ($P<0.05$) between the cells groups. Further characterization using western blot analysis confirmed the presence of ALIX, and the absence of GRP78 protein in the EVs. In addition, transmission electron microscopy (TEM) showed EVs in the expected shape and size (<150nm). To isolated TC cells (n = 4 lines), we carried out in vitro fertilization, and Day 8-hatched blastocysts were single cultured on Matrigel (1.5 mg/mL). EVs obtained from the culture media showed an average size of 167.77 nm and concentration of 2.70 x 108 particles/mL. The EVs size and concentration of particles were similar ($P<0.05$) among the lineages. To in vitro simulate the maternal-embryonic crosstalk and investigate if cells from one source can modulate transcripts in the target cells, we treated the TC with EVs from the endometrial cells, and the endometrial cells were treated with EVs from the TC. Cells were collected and stored at -80 °C and they will be submitted to RNAseq for gene expression analysis. In this project we intend to better understand the internalization and modulation of EVs produced by endometrial and trofectoderm (TC) cells cultured in vitro and their effects in the trasncryptome in each target cell.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Determination of pregnancy loss at different moments of early gestation in dairy cattle subjected to TAI

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Resumo

The objective of this study was to evaluate the occurrence of pregnancy loss in dairy cattle using different pregnancy biomarkers. The study was conducted in two commercial dairy farms. Holstein cows (n=140) and heifers (n=32) were subjected to a hormonal synchronization protocol and TAI (D0). At D21 post-TAI, blood samples were collected for peripheral blood mononuclear cells (PBMC) isolation and for assay of plasma progesterone by radioimmunoassay kit (MP Biomedicals), and corpus luteum (CL) blood perfusion was evaluated by Doppler ultrasonography. An active CL was considered when the blood perfusion was >25% and progesterone concentrations were >1 ng/mL. Plasma samples collected on D25 were assayed for pregnancy-associated glycoproteins (PAGs) using a commercial ELISA kit (Ruminant pregnancy test kit, IDEXX® laboratories). The abundance of interferon-tau stimulated genes (ISG15 and RSAD2) in PBMC was determined by RT-qPCR and normalized to GAPDH and PPIA. Confirmatory pregnancy diagnosis was performed on D32 and D60 days post-TAI by B-mode ultrasonography. Statistical analyses were performed using Chi-Square of SPSS software. The pregnancy biomarkers were used to categorize the females that have undergone late luteolysis (LL - Non-pregnant females at D32, but with an active CL at D21, and considered non-pregnant by abundance of ISGs on D21 and PAG's test on D25); early embryonic mortality from 21 to 25d (EEM - Non-pregnant at D32, but with presence of an active CL and considered pregnant by ISG abundance on D21, and non-pregnant by PAG's test on D25); late embryonic mortality from 25 to 32d (LEM - Non-pregnant at D32, but with presence of an active CL and considered pregnant by ISG abundance on D21 and PAG's test on D25); and late pregnancy loss from 32 to 60d (LPL - Pregnant at D32 but non-pregnant on D60). Cows were also evaluated if had previous postpartum issues (metritis, repeat breeder [>3 inseminations], retained placenta, abortion or stillbirth). A lesser rate of LL was observed in heifers (P=0.02) than cows (6% [2/32] vs. 26% [36/140]); however, no difference (P>0.1) was found for EEM (6% [2/32] vs. 9% [13/140]), LEM (11% [4/32] vs. 6% [9/140] and LPL (0% [0/32] vs. 6% [9/140]). The pregnancy rate on D60 did not differ (P>0.1) in cows with postpartum issues (18% [15/84]) or not (27% [15/56]). Unhealthy cows had greater (P<0.05) rate of LEM (11% [9/84] vs. 0% [0/56]) and lesser rate of LPL (2% [2/84] vs. 12% [7/56]), but no difference was observed for LL (25% [21/84] vs. 27% [15/56]) and EEM (10% [8/84] vs. 9% [5/56]). In conclusion, lactating dairy cows had a greater occurrence of LL than heifers, indicating a possible extension of CL life-spam without involving interferon-tau stimulus. Reproductive postpartum issues affect the occurrence of pregnancy loss in dairy females, especially in the early stages of pregnancy.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Hair color on production and reproduction of Angus heifers

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Resumo

The perspective of global temperature increase makes essential to identify more adapted individuals. Thus, hair coat (HC) is evaluated in genetic selection programs. This study was designed to determine the influence of hair color (red or black) on HC, body weight, body condition score (BCS) and puberty in Angus heifers (52 black and 41 red) from 15 to 18 month-old, raised in Dom Pedrito-RS. Animals were evaluated three times, with 45 days interval, from October 2021 to January 2022. At each evaluation heifers were weighed, evaluated for BCS (1-5), classified according to HC (HC1 - short, fine and flat hair, HC2 - intermediate and HC3 - long, thick and woolly hair), and evaluated to determine the reproductive tract score - RTS (1-3 = prepubertal and 4-5 = pubertal). Puberty data were evaluated by Chi-square, body weight data by analysis of variance, and BCS data were evaluated by the Kruskal-Wallis test. Means were compared by Fisher's test. The hair color had no influence on the body weight of black (270.38kg, 313.24kg, 320.40kg) and red (260.72kg, 306.63kg and 310.77kg) heifers in the first, second and third evaluation, respectively. However, the HC was associated with ($P<0.05$) this characteristic at the first evaluation (HC1=283.63kg, HC2=264.09kg and HC3=248.94kg). In the second evaluation, the body weight of HC1 animals (324.31kg) was higher ($P<0.05$) than HC2 (309.57kg) and HC3 (295.93kg). At the third evaluation body weight was different ($P<0.05$) for HC1 (333.72kg), HC2 (315.09kg) and HC3 (297.95kg). The hair color did not influence the BCS ($P>0.05$), but the HC was associated in the last two evaluations ($P<0.05$). In the second evaluation, the HC3 group had lower BCS than the others ($P<0.05$). In the third evaluation, the HC1 animals had higher BCS than the HC3. HC was not associated with RTS during the evaluation period, but the hair color had influence in the first and second evaluations. None of the red and 9.6% of the black animals were classified as 4-5 in the first RTS evaluation and RTS 4-5 was identified in 86.5% of the black and 61% of the red animals ($P<0.05$) in the second evaluation. Whereas, at the third evaluation, the percentage of animals with RTS 4-5 was similar ($P>0.05$) between black (88.5%) and red (73.2%) heifers. Our results show that, although the HC is associated with body weight and BCS of Angus heifers, it is not associated with the percentage of cyclic animals. On the other hand, the higher percentage of cyclic black hair heifers at 15 and 16.5 month-old, is probably due to a greater selection pressure on these animals, since their numbers in the general Angus herd are higher than the red animals. The HC was not associated with cyclicity, but the body weight and BCS of animals with HC 1 and 2 was higher. To better understand the effect of hair color and type on cyclicity, new studies need to be performed, aiming to increase the number of animals evaluated.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Nitric oxide acts as an epigenetic regulator of histone H3K9 acetylation in oviduct cells in luteal and follicular phase of estrous cycle

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Resumo

Reproductive events that occur in the oviduct depend on signaling molecules, e.g. nitric oxide (NO). The NO, a short-lived free radical, has a variety of cellular effects, including alterations in mitochondrial metabolism and participation in reproductive processes such as fertilization and preimplantation embryo development. Recently, NO has also been associated to Histone Deacetylase 2 (HDAC2) function in neurons, resulting in altered pattern of histone acetylation, with consequences to the gene expression. Thus, this study aimed to analyze whether differences in the NO availability in the bovine oviduct epithelial cells (BOEC) lead to changes in the mitochondrial metabolism and in the histone H3K9 acetylation profile (H3K9ac). BOEC were collected from a slaughterhouse from animals at luteal (L) or follicular (F) phases (set by ovarian morphology). In total, 12 animals were selected and divided in three replicates. In each replicate, BOEC of the same estrous cycle phase (n=2 animals/phase) were pooled and cultured in DMEM + 10% FBS (38°C, 5% CO2, high humid) until 70% confluence, moment when the cells were submitted to the treatments. Cells were treated with S-Nitrosoglutathione (GSNO), a nitric oxide donor, in three concentrations: zero (control), 100 (GSNO100), and 500 µM (GSNO500). Therefore, six groups were analyzed according to the treatment and the estrous cycle phase: control follicular (CF), control luteal (CL), GSNO100F, GSNO100L, GSNO500F, and GSNO500L. The mitochondrial membrane potential (MMP) was analyzed by fluorescent probe and the H3K9ac levels by immunocytochemistry, both analysis were done at 0h (before treatment), 4h, 48h, and 96h after addition of GSNO (0, 100, and 500 µM). Microscopy images were processed by the Fiji package and the data analyzed by the GraphPad Prism software (Kruskal-Wallis test for non-parametric data and Tukey test for parametric data, $p<0.05$). The MMP remained constant in all samples, except for GSNO500F and GSNO500L, which was higher than CF and CL, respectively, at 48h of incubation. The H3K9ac profiles were distinct in each estrous phase. In the F phase, the control group assumed a V shape profile as time passes (high at 0h, low at 4h, lower at 48h, and up at 96h similar to 0h), while the GSNO treatments showed no effect at 4h, but both GSNO100F and GSNO500F were higher than CF at 48h, and GSNO500F were lower than CF at 96h. In the L phase, the profile of the control group was a gradual decrease over time (0h > 4h = 48h > 96h), while the GSNO100L treatment led to higher H3K9ac levels at 4h and 48h than their respectively control groups and the GSNO500L treatment had a later effect increasing the H3K9ac levels at 48h and 96h compared to their respectively control groups. In conclusion, NO acts to increase H3K9ac levels in oviductal cells, which might occur by inhibiting HDACs, however this modulation seems to be dependent on the estrous cycle phase.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Effects of Zona-Free Culture on IVF bovine embryos and hemiembryos.

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Resumo

In recent years, IVF have become one of the most applied biotechnologies in livestock production. Monozygotic twin production has been successful in bovine species, by removal of the Zona Pellucida (ZP) and blastomere disaggregation at two cells stage. Since monozygotic twins are identical, it is possible to duplicate the number of embryos of superior genetic value from a single embryo. The objective of this work is to evaluate the effect of the ZP-Free embryo culture and blastomere separation in blastocyst development rate. Ovaries were collected from slaughterhouse and transferred to laboratory in sterile physiologic solution. COCs were aspirated from follicles of 2-8mm using 18G needles. COCs were searched in Tyrode's Album Lactate Pyruvate Hepes (TALP-H) with 1% Penecillin-Streptomycin Fungizone (Gibco, USA), then maturated in vitro in Tissue Culture Medium 199 (TCM 199, Gibco, USA) 100µl drops under mineral oil 22 hours, in humidified air at 38,5ºC (Gibco, USA). Matured COCs were co-incubated in contact with thawed bull sperm, at $16x10^6$ spz/ml concentration, diluted in Sperm Wash Solution and Sperm Dilution Solution in the same proportion, in 100µl drops under mineral oil 5 hours. Presumptive zygotes were deposited in 100µl of TALP-H solution, and agitated 1 minute to remove the cumulous cells and spermatozoids adhered. Presumptive zygotes were then separated in two groups. One group was transferred to 50µl drops of Synthetic Oviductal Fluid (SOF), with 2.5% of FBS, 7 days and 38.5ºC and 5% O_2 under mineral oil. Cleavage was evaluated at 30 hours post IVF. The other group was transferred to 100µl TALPH drops covered in mineral oil to remove the ZP, with 10µl of protease 1 minute. ZP-free embryos were washed in TALP-H and individually cultured in microwells in SOF medium under mineral oil and 38,5ºC and 5% O_2. 30 hours post IVF, cleavage rate was evaluated. Half of the ZP-free embryos were selected randomly. Two-cells embryos were washed in TALPH and transferred to 50µl Drops of Dulbecco's phosphate-buffered saline (DPBS) medium (Gibco, USA), with no Ca_{2+}, no Mg_{2+}, and 20% of FBS covered in mineral oil. Blastomeres were separated by gently pippeting and individually cultured in microwells under same culture conditions. Blastocyst rate were evaluated at Day 7 of embryo culture. Blastocist rate from Control IVF Embryos, vs ZP-free embryos and hemiembryos were compared by Chi-Square Test ($P<0.05$). Our results suggest that both hemiembryos and ZP-free embryos had reduced blastocyst rate (13% and 10%, respectively), versus Control blastocyst rate (24%). The impairment in ZP-free embryo development could be caused by ZP removal procedure or ZP-free embryo culture. In conclusion, blastomere disaggregation for monozygotic twinning does not affect the blastocyst rate, apart from the ZP-free culture procedure. Improving ZP-free embryo culture could increase the total embryo production of monozygotic twins.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

IVM SYSTEM ALTERS KDM4C TRANSCRIPT LEVELS IN BOVINE OOCYTES

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Resumo

Maternal mRNAs play essential functions in early embryo development. During oocyte maturation, ZFP36L2 binds AU-rich transcripts, directing them to degradation and remodeling mRNA stores. Our previous data showed a difference in ZFP36L2 mRNA levels between oocytes matured in vivo vs in vitro. This study aimed to evaluate ZFP36L2 expression and activity in bovine cumulus-oocyte complexes (COCs) submitted to different in vitro maturation (IVM) protocols. Grade I and II COCs from 2-8 mm follicles were distributed into 3 IVM groups, containing 50 COCs per well and 400µL of the following IVM media: conventional IVM for 19 h [C-IVM; TCM199 Earle's salt and L-glutamine, 2.2g/L sodium bicarbonate, 50µg/mL gentamicin, 0,2mM sodium pyruvate (base medium) plus 0,5µg/mL FSH, 50mg/mL hCG e 10% fetal calf serum]; "physiological" IVM for 19 h (Ph-IVM; base medium plus 10ng/mL IGF-1, 100ng/mL AREG, 10-2 IU/mL rhFSH, 5µg/mL 17β-oestradiol e 150ng/mL progesterone); pre-IVM (PM-IVM; base medium plus 500ng/mL 17β-oestradiol, 50ng/mL progesterone, 50ng/mL androstenedione, 10-4 IU/mL rhFSH and 100nM NPPC) for 9 h followed by Ph-IVM for 19 h. Samples were collected at 0 h (germinal vesicle - GV), end of pre-IVM (PM-IVM 0 h), 9 h, and 19 h of IVM only oocytes in Metaphase II (MII) were collected. Oocytes and cumulus cells were separated, frozen and stored. Ten pools of 50 oocytes from 9 h IVM groups and 6 pools of MII oocytes were used for ZFP36L2 quantification by western blotting (WB). Twenty COCs/group were collected at 0 h and 9 h IVM, fixed and immunostained with ZFP36L2 antibody for protein detection, Hoechst 33342 for nuclei observation, and Alexa Fluor 647 Phalloidin for transzonal projections (TZP) visualization. Seven pools of 10 oocytes were analyzed for expression of ZFP36L2 and its target genes KDM4C, KDM5A, CCNE1, FBXO5 e FBXO43 by RT-qPCR. MII rates were analyzed by Chi-square test followed by Fischer's exact test. WB results were submitted to ANOVA followed by Tukey's test. Gene expression data were transformed by ΔΔCT and normalized by housekeeping genes ACTB e PPIA. Results considered 5% significance level. MII rates were different between PM-IVM (68.37b%) vs C-IVM (73.19a%) ($p=0.004$) and Ph-IVM (79,13a%) ($p<0.0001$). ZFP36L2 protein levels were similar among groups at 9 h IVM (C-IVM: 1048; Ph-IVM: 1059; PM-IVM: 1132; arbitrary units) and MII oocytes (C-IVM: 558; Ph-IVM: 674; PM-IVM: 677). PM-IVM was efficient to prevent GVBD and sustain TZP integrity for 9 h, visually similar to GV. No differences were observed for gene expression at 0 h (GV vs PM-IVM 0h). KDM4C was downregulated ($p=0.0078$) in oocytes from the PM-IVM compared to C-IVM (~43%) and Ph-IVM (~26%) at 9h IVM, and upregulated (1.5 fold) in MII oocytes from the Ph-IVM vs PM-IVM ($p=0.0137$). These data indicated that the IVM protocol did not alter ZFP36L2 mRNA and protein levels, but impacts KDM4C expression in oocytes.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Effect of FGF2, LIF, and IGF1 supplementation on pregnancy success following embryo transfer of IVP embryos

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Resumo

Culture environment during IVP can affect embryo phenotype and pregnancy outcomes, making culture modifications a logical approach for improving embryo competence. Previously, the addition of the growth factors FGF2 (40ng/ml), LIF (20ng/ml), and IGF1 (20ng/ml), termed FLI, to the culture medium improved bovine embryo development, and re-expansion following cryopreservation (39% to 82%). The objective of this study was to investigate the survival of cryopreserved FLI treated embryos at days 15 and 30 and evaluate conceptus transcriptomes. Embryos were produced using abattoir-derived oocytes, fertilized using standard procedures, and cultured to the blastocyst stage with or without FLI (+/- FLI). Embryos with a quality grade of 1 (6-1) were loaded into straws with ethylene glycol + sucrose and cryopreserved by slow-rate freezing. For experiment 1, 65 -FLI and 65 +FLI embryos were transferred into non-lactating recipient beef cows (n = 26 / 5 embryos each) 7 days after the last GnRH of a 5-day CO-Synch protocol. Eight days later, females were euthanized, uteri were collected, flushed, and conceptuses were recovered and flash frozen. For a subset (n = 4 per treatment) whole transcriptome analysis was performed using the NovaSeq 6000 (NGS platform of Illumina). Sequencing depth was 50 million reads per sample. After quality control, transcriptome of samples was aligned to the cow genome using Hisat2 and FeatureCounts was used to determine the read counts per gene. EdgeR was used to identify differentially expressed genes (FDR < 0.05). In experiment 2, a single frozen-thawed embryo was transferred to recipient females (n = 130) 7 days following detection of estrus. Pregnancy diagnosis was performed on day 30 using transrectal ultrasonography. Data for embryo length and average embryos recovered were analyzed by ANOVA using the GLM procedure of the Statistical Analysis System (SAS V9.4). Data for embryo recovery and day 30 pregnancy were analyzed by logistic regression using the Glimmix procedure. In experiment 1, there was no difference (P > 0.05) in conceptus recovery (-FLI 33.8% ± 5.87 vs +FLI 32.3% ± 5.8) or average conceptus length (-FLI 3.33cm ± 0.73 vs +FLI 4.18cm ± 0.75). There were 32 differentially expressed genes, 23 upregulated and 9 down regulated in the +FLI group compared to -FLI. Genes were involved in interferon signaling, prostaglandin synthesis, and the MAP kinase pathway. The +FLI group had increased expression of genes involved in trophoblast formation. In experiment 2, pregnancies per ET were 30.88 ± 5.6% in the -FLI group and 30.65 ± 5.9% in the +FLI group (P = 0.98). We conclude that embryos cultured +/- FLI and cryopreserved by slow-rate freezing have similar developmental competence up to day 30 of pregnancy. Differences in gene expression show an effect of FLI on conceptus signaling during elongation.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Role of PI3K/AKT/PTEN pathway inhibitors during IVM of mammalian oocytes: a systematic review

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Resumo

Modulation of phosphoinositide 3-kinase/protein kinase B/phosphatase and tensin homolog (PI3K/AKT/PTEN) pathway in mammals yields mixed results. Understanding its regulation can be a powerful tool for improving IVP. This systematic review aimed to map the evidence of mammalian PI3K/AKT/PTEN pathway modulation during IVM, to assess its effects on meiosis resumption/germinal vesicle breakdown (GVBD) and progression to metaphase II (MII), as well as its impacts on embryo development and quality. Three databases were fully searched, and 20 articles were considered suitable after screening. Ten, six, and four articles were reported in swine, bovine, and murine, respectively. A total of 48 IVM studies were identified considering different experimental conditions within the same article, among which 11 evaluated blastocyst yield in swine and bovine. Three PI3K inhibitors [3MA, Wortmannin (Wo), and LY294002 (LY)] and one AKT inhibitor (SH6) were investigated, accounting for 8, 29, 46, and 17% of the studies, respectively. The GVBD and MII rates were categorized as exhibiting positive effects (ability to inhibit) in 44% and 81% of the studies, respectively. In swine, 21 studies analyzed the supplementation of LY (52%), Wo (14%), 3MA (19%), and SH6 (14%) during IVM. Regarding GVBD, the addition of 5 x 10-6 to 7.5 x 10-5 M LY, 10-9 to 10-6 M Wo, and 10-2 M 3MA yielded a positive effect. Progression to MII was categorized as positive when 1 x 10-6 to 5 x 10-5 M LY, 10-5 M Wo, 10-2 M 3MA, or 5 x 10-5 M SH6 was added. In bovine, 17 studies analyzed the addition of LY (23%), Wo (65%), and SH6 (12%). The addition of LY (10-4 to 7.5x10-5 M) or Wo (10-8 to 10-6 M) was not able to inhibit GVBD, but a positive effect was shown in MII rate with 10-4 to 7.5 x 10-5 M LY, 10-8 to 10-6 M Wo, and 5 and 7.5 x 10-5 M SH6. In murine, 10 experiments were extracted in which 70 and 30% applied LY and SH6, respectively. Effects over GVBD and MII rates were positive, respectively, in 70% and 50% of the studies, and were dependent on media composition and pathway promoters, such as EGF and FSH. Post-IVM assessments were described in swine and bovine, and similar-to-control rates were seen. However, the addition of 2 x 10-8 M Wo in bovine was able to enhance cleavage and blastocyst rates. In this sense, two applied strategies allowed similar and greater than control rates: reduction of PI3K activity and temporary blockage of GVBD. Thus, GVBD and MII pathway regulation seems to depend on the species, inhibitor, concentration, and media supplementation. While in bovine, GVBD seems to be pathway independent, in swine and murine it is not well established. However, MII is highly controlled by the pathway on both bovine and swine. These data highlight the important roles of PI3K/AKT/PTEN pathway in mammals, the strategies, and the potential for improving IVP efficiency, underlining this pathway could be well explored in further studies.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Impact of semen variability on IVP efficiency

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Resumo

In IVP the semen quality and its competence to fecundate and produce a healthy embryo is often not explored. The aim of this work was to study the impact of semen variability on IVP efficiency and its relationship with semen quality analysis. Frozen commercial samples of 5 Braford bulls were analyzed. Two experiments were performed, in the first after straw thawed each semen sample was divided, an aliquot was destined to IVP and the other for quality evaluation. To IVP, COCs from slaughterhouse ovaries were subjected to IVM in TCM 199 for 22 h. IVF was performed in BO medium with 16x106 spermatozoa/ml/5h with complete sample. IVC was performed in SOF supplemented with 2.5% FCS for 7 days at 38,5 °C and 6,5% CO2 (N=20). To evaluate individual development 20 presumptive zygotes of each treatment were individually cultured in WOW system, a conventional drop culture was maintained as control. Cleavage and blastomeres number were registered at 26 and 48 hours after IVF and blastocyst rates were determined at day 7. To evaluate semen quality, we registered live and abnormal spermatozoa % (AS) at 0h and progressive motility (PM) and individual vigor (IV) at 0 and 2h. In the second experiment acrosomal reaction (AR) was assessment using Gimesa stain immediately post thawed and after BO incubation at 0; 2.5 and 5 h. Pearson correlation was evaluated between cleavage and blastocyst % and with semen quality variables, AR % were analyzed using Chi square test. Cleavage % at 26 and 48h were statistically lower in two of samples evaluated (A:48-58a, B:10-18b, C:43-73a, D:50-65a, E:3-5b). Blastocyst % were statistically different between samples (A:13a; B:0b; C:30a; D:33a; E:0b). There was no detected correlation between cleavage at 26 and 48h and blastocyst rates. There were no differences in blastocyst rates with control group. Quality analyses showed similar values of live; AS, PM % and IV at 0 h (A:38-9-58-4; B:35-12-43-3; C:34-12-65-4.2; D:40-11-65-4.5; D:42-12-56-3.8). After 2h of incubation were registered in sample 2 100% of losses to % of motility and vigor and similar losses among the other ones (A:49-54, B:100-100, C:23-28, D:51-78, E:49-61). There was no founded correlation between semen quality variables and embryo %. In the second experiment the % of AR increased during the incubation, get the max level at 2,5 h. (post-thawed: 4.33c; 0h:9.17b; 2.5h:15.67a; 5h:14.21a). Samples B and C needed more time of incubation to acquire the max level of acrosome reaction (A: post-thawed: 3.17c; 0h:8.0b; 2.5h:17.17a; 5h:13.17a-B: post-thawed: 4.0c; 0h:8.67b; 2.5h:10.67b; 5h:16.0a -C: post-thawed: 2.5c; 0h:6.0b; 2.5h: 5.5b; 5h:11.0a -D: post-thawed: 5.83c; 0h: 13.5b; 2.5h:19,5a; 5h:15.0b -E: post-thawed: 4.33b; 0h:6.5b; 2.5h:15.33a; 5h:12.67a). In conclusion, semen denotes an important role in the embryo IVP system, affecting since the capacitation, fecundation until blastocyst development and its performance in not predictable by quality analyzes.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Use of comparative proteomic analysis to investigate the effects of near physiological temperature of 37.5 ºC on the in vitro maturation of bovine oocytes

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Resumo

The temperature inside the preovulatory follicles of cows *in vivo* is approximately 1 ºC lower than the rectal temperature. However, bovine oocyte *in vitro* maturation (IVM) protocols defined 38.5 ºC as the standard temperature, mainly based on rectal temperature. In this study, we evaluated the effect of a temperature reduction (37.5 ºC, treatment group) during IVM in relation to that used routinely (control group) on the proteomic profile of oocytes. After the IVM, the nuclear maturation rate and expansion degree of cumulus cells (CC) (30 COCs per group with 21 replicates) were evaluated by observation of the first polar body and using a subjective scoring method (0-4), respectively. The total nitrite concentration in the culture medium (5 per group with two replicates) were measured by the Griess method. The data were evaluated by analysis of variance and the Student t-test without transformation, at 5% probability. The proteomes of the pools (500 maturated oocytes per group with three replicates) of oocytes were characterized by high-performance liquid chromatography-mass spectrometry. The differentially accumulated proteins (DAPs) were analyzed using ProteinLynx Global SERVER (PLGS) v.3.02, and data enrichment was performed using several bioinformatics tools, allowing the identification of signaling pathways, gene ontology classification, predicted protein-protein interaction analysis and putative gene regulation by miRNAs. We did not observe any difference between the groups in the nuclear maturation analyses, expansion of CC and total nitrite concentration in the culture medium (P>0.05). The comparative proteomic analysis identified a total of 806 proteins, of which 7 were up-regulated and 12 down-regulated (treatment/control comparison). In addition, 12 proteins were found exclusively in the control group, whereas 8 were identified only in the treatment group. The DAPs occurred in the nucleus, cytoplasm, plasma membrane, mitochondria, cytoskeleton and undescribed locations. In the hallmark pathway, it was possible to observe the presence of metabolic pathways such as MYC V1 targets, oxidative phosphorylation, MTORC 1 signaling, fatty acid metabolism and glycolysis. In the KEGG pathways analysis, the glycolysis/gluconeogenesis, actin regulation in the cytoskeleton, citric acid cycle and pyruvate metabolism pathways were enriched. Among the 12 down-regulated proteins, six proteins are known to have their enconding gene regulated by miRNAs. Using miRNet network analysis, we identified 43 bovine miRNAs that regulate the expression of these genes (DES, HMOX2, KRT75, FARSA, IDH2, CARHSP1). This is the first comparative proteomic study of bovine oocytes submitted to different temperatures during IVM. We conclude that the proteome of oocytes during IVM is thermomodulated, with significant effects in cell metabolism.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Impact of Sperm Sexing Technique on Quality of In Vitro Produced Bovine Embryos

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Resumo

Although sperm sexing technology has been widely studied, many evidences confirms this kind of environment stressors have serious impacts on reproduction, although its influence on embryonic quality and/or offspring is not very well stablished in domestic animals. Therefore, here was proposed to evaluate the paternal effect on IVP embryos, using flow cytometry sperm sexing as a model. For this purpose, we used conventional (CV) and sexed male (SX-Y) and sexed female (SX-X) semen from 5 different Nellore bulls. Oocytes obtained from slaughterhouse ovaries were submitted to IVM for 24 hours, then were inseminated (D0) with CV and sexed semen (SX-Y and SX-X) from the 5 different bull semen. To eliminate sexed effect but preserve the sexing procedure effect SX-Y and SX-X were pooled to form the sexed (SX) sample. On D1, possible zygotes were transferred to culture medium – SOF with 0.4% BSA, where they remained for 8 days. Cleavage rates at D2, and blastocyst rates at D6, D7 and D8 were evaluated. At D8, expanded blastocyst stage embryos were storage for gene expression analysis. Five genes (OCT4, NANOG, Fematrin-1, DNMT3A, TET1) were chosen to be evaluated by qPCR. Blastocysts rates data were analyzed by Chi-square, and for individual bull rates effect and gene expression ANOVA was used, considering $P \leq 0.05$ for all analysis. Cleavage rate at D2 (77.13% x 70.24%) and blastocyst rate at D6 (6.56% x 2.61%), D7 (21.2% x 9.9%) and D8 (26.64% x 14.1%) were higher ($P<0.05$) for CV than SX. Analyzing developmental kinetics, CV semen had greater ($P<0.05$) percentage of expanded blastocysts than hatched blastocysts on D7 (11.84% x 0.3%) and D8 (14.04% x 0.96%), while SX semen had this difference only on D8 (9.41% x 0.65%). When comparing embryo development rates between semen CV and SX for each bull (B1 – B5), no differences were observed at D2 and D6. However, B2 and B3 presented superior blastocysts rates on D7 and D8 for CV (B2: 22.3%, D7 and 30.4%, D8; B3: 32.9%, D7 and 42.0%, D8) than their SX counterparts (B2: 6.0%, D7 and 9.3%, D8; B3: 16.3%, D7 and 20.6%, D8). Regarding to gene expression evaluation, only NANOG presented difference ($P=0.002$) between CV and SX treatments. Results evidenced that sexing procedure affected bovine embryos production, embryo kinetics and gene expression. In addition, the effect were influenced by the sire used for IVF step. To supplement the founding evidences and determinate embryo quality markers, additional analysis accomplishments are necessary.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Comparative response to bovine conceptus and accuracy as early pregnancy predictors of interferon-tau stimulated genes, and cytokines in immune cells

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Resumo

We aimed to compare: 1) the expression of interferon-tau stimulated genes (ISG) and cytokines in PBMC (mononuclear) and PMN (polymorphonuclear) cells stimulated by recombinant interferon-tau (rIFNT) or uterine flush (UF) from pregnant cows (in vitro Study); and 2) the recent identified biomarkers (RSAD2 and IFI44) for assessment of accuracy (Ac) as early pregnancy predictors (in vivo Study). In the in vitro Study, PBMC and PMN were isolated from blood of non-pregnant Nelore cows (n=9) between 10-12 days post-ovulation. For Exp. 1, cells were treated or not (control) with 100 ng/mL rIFNT and incubate for 24 (PBMC) or 3 h (PMN) at 37°C in 5% CO2. For Exp. 2, cells were incubated in UF from cows in day-18 of diestrus phase (control) or from day-18 pregnant cows (UF-Conceptus) for 12 (PBMC) or 3 h (PMN). Gene expression was determined by RT-qPCR. For in vivo Study, Nelore females (nuliparous, n=103; primiparous, n=53; pluriparous, n=91) were submitted to timed-AI on day 0. On D20, PMN was isolated from blood and Doppler ultrasonography was done to evaluate CL. Data were analyzed by ANOVA using the PROC MIXED procedure (SAS) and ROC curves to determine the Ac of pregnancy predictors on D20 (ISGs and Doppler). Expression of all ISGs was greater (P<0.05) in both cells treated with rIFNT and UF-Conceptus than its controls. Fold change in rIFNT-treated PBMC was greater (P<0.05) for ISG15 and RSAD2 than for IFI44, whereas, RSAD2 had the greatest fold change in PMN. For PBMC and PMN treated with UF-Conceptus, fold change was greater (P<0.05) in ISG15 and RSAD2. Expression of IL1β was lesser (P<0.05) in PBMC and PMN treated with UF-Conceptus; however, no difference (P>0.1) was observed between the IFNT and controls groups in both cell types. For IL10 expression, no difference was observed between treatments in both cell types. For in vivo Study, RSAD2 and IFI44 expressions were greater (P<0.05) in pregnant compared to non-pregnant females in all parity categories, but expression was greater in nulliparous than pluriparous cows. The two classic ISGs evaluated (ISG15 and OAS1) and the unusual ISGs (RSAD2 and IFI44), were significant (P<0.01) predictors of pregnancy in all parity categories; however, the RSAD2 and IFI44 resulted in superior Ac. The Ac (%) in nulliparous, primiparous and pluriparous cows were, respectively, for RSAD2, 79, 90 and 92, and for IFI44, 86, 82 and 90. All gene combinations were tested and the best association for increase of Ac (92.7%) and reduction of false negative results (0.9%, 2/233) was obtained when pregnant animals were considered if one of the four ISGs were stimulated in females with an active CL (>25% of luteal blood perfusion) on D20. In conclusion, the ISG15 and RSAD2 were the most stimulated ISG in PBMC and PMN, indicating that an association of various classic and non-classic ISGs, can be used to improve the pregnancy prediction on day 20 in beef females with an active CL.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Low-PAG pregnancies are characterized by disturbances at the maternal-conceptus interface and impaired placenta development

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Resumo

The objective of this study was to evaluate transcriptomic differences in reproductive tissues of beef heifers with high vs. low Pregnancy Associated Glycoproteins (PAG) concentrations carrying viable pregnancies during the attachment and early placentation periods. Bos indicus beef heifers (n=25) were subjected to embryo transfer on day 7 with IVP embryos, and pregnancies were confirmed at slaughter on days 25 (n=8) and 36 (n=8). Samples from the endometrium (END) and trophectoderm (TE) were collected for RNA isolation using the RNeasy kit (QIAGEN; Hilden, Germany). Plasma concentrations of PAGs were determined via a validated ELISA approach and the females were classified into groups of high and low PAG (6.8±0.7 (n=4) vs. 0.8±0.7 (n=4) on D25 and 15±2.1 (n=5) vs. 4±1.2 (n=3) ng/mL on D36; P<0.05). An Illumina platform was utilized for RNA sequencing. Differentially expressed genes (DEGs) between groups and by tissue were determined using edge-R package from R. On D25, 106 DEGs were reported in the TE, and 56 in the END between the high and low PAG groups. On D36, 1136 genes were differentially expressed in the TE, and 383 in the END. Gene ontology analyses revealed downregulation of processes associated with angiogenesis, and embryonic, and placenta development on D36 in both TE and END for the low PAG group, although these responses were not evident on D25. In the low PAG groups of both D25 and D36, the most significant pathways and biological processes in the TE were associated with extracellular matrix (ECM) remodeling and organization, represented by an up-regulation of remodeling proteases (e.g., MMP2, MMP9, MMP14, ADAMTS2, and ADAMTS8) and ECM proteins (collagens, elastin, and laminin). In the END, however, these processes were significantly downregulated in the low PAG groups. Attachment and adhesion markers, such as integrins and glycans, were also up-regulated in the TE of low PAG groups on both days. Although PAG concentrations were significantly different in high vs. low PAG groups, on D25 and D36 PAG genes were not differentially expressed. In conclusion, heifers with low PAG in peripheral circulation have significantly different gene expression profiles in the developing conceptus and endometrium. Markers of attachment, ECM remodeling, and invasion were significantly up-regulated in the TE, but not in the END of the low PAG heifers, demonstrating an abnormal fetus-maternal contact initiated on D25, which may have resulted in poor embryonic and placenta development on D36. Disturbances at the maternal-conceptus interface could be impairing PAGs from reaching the maternal peripheral circulation since no differences in PAG genes were found between high vs low PAG groups. Therefore, PAGs could be indicative of conceptuses with different capacities to maintain pregnancy. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2017-67015-26457 from the USDA National Institute of Food and Agriculture.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Day 7 in vivo- or in vitro- produced bovine embryos induce distinct molecular alterations in the endometrium

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Resumo

The first interactions between embryo and endometrium are important for pregnancy establishment and maintenance. On day 15, conceptuses produced from different biotechnologies altered the expression of interferon–tau dependent and independent genes in endometrial cells (Mathew et al., 2019. Biol Reprod. 100:365-380). However, endometrial molecular modifications caused by a single day 7 in vivo or in vitro blastocyst after the embryo transfer are still elusive. Thus, we hypothesized that a single day 7 in vivo or in vitro-derived bovine embryo modify endometrial global transcriptomic response when co-cultured without contact with endometrial explants. For this, Day 7 embryos were produced in vivo or in vitro. The endometrial explants were collected from the uterus of cows (n=3) previously synchronized to be on day 7 of the estrous cycle. Endometrial explants from the same uterus were cultured with medium (without embryo; EE), or a single day 7 in vivo–produced bovine embryo (EE-AI), or a single day 7 in vitro-produced bovine embryo (EE-IVF). The co-cultured was performed using inserts with pores of 0.4 µm size to retain the embryo directly above the endometrial surface for 24 hours. Total RNA extraction from endometrial explants was done using miRNeasy Mini Kit (QIAGEN) following the manufacturer's instruction. The RNA library preparation was performed using Illumina TruSeq Stranded mRNA Sample Prep kit. The sequencing was performed in 1 lane of Hiseq 2500 V4 (2x100pb). Compared to EE, 273 differently expressed genes (DEGs) were identified in EE-AI and 409 in EE-IVF (P-adjust.≤ 0.1 and log2FoldChange>0.6) groups. Of these, 142 DEGs were expressed in EE-AI and EE-IVF groups, among these DEGs, some traditional IFNt responsive genes (ISG15, MX1, MX2, RSAD2, and OAS1Y) were more expressed in these groups than EE. The tops three pathways identified in enriched KEGG analysis were influenza A, measles, and herpes simplex infection (P-adjust.≤ 0.1). Between the EE-AI and EE-IVF groups, 156 genes were differentially expressed (P-adjust.≤ 0.1). The top five enriched pathways identified were calcium signaling pathways, ABC transporters, renin secretion, focal adhesion, and complement and coagulation cascades (P-adjust.≤ 0.1). Of these 156 DEGs, 87 were exclusively altered when compared EE-AI with the EE-IVF group. The GO analyses identified steroid hormone mediated signaling pathway, and positive regulation of JNK cascade as the biological processes associated with these 87 DEGs (P-value.≤ 0.1). In conclusion, the present study showed that the presence of a single day 7 blastocyst was able to induce DEGs in endometrial explants in an origin-dependent fashion (in vivo or in vitro). Curiously, we identified endometrial DEGs modulated by the embryo presence, demonstrating that these DEGs possibly have an important role during the first embryo-maternal interactions.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Effects of forskolin supplementation at different stages of IVP on the preimplantation development of bovine embryos

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Resumo

Lipid accumulation is commonly observed in IVP embryos. It is used as a parameter to assess the embryonic quality and has a direct impact on cryosurvival. Supplementing culture media with metabolic regulators is a promising strategy to improve cryopreservation results, however, the ideal period time to supplement these agents in IVP has not yet been defined. The objective of this work was to evaluate the effects of forskolin (FO), a lipolytic agent that increases the levels of cAMP in cells, on cleavage and blastocyst (D7) rates, aiming at future increases in cryopreservation rates, by reducing lipid content. Oocytes obtained from slaughterhouse ovaries were in vitro matured (TCM199 with 10% FCS, hormones and sodium pyruvate) and fertilized (Talp-FIV with 0,6% BSA). Presumptive zygotes were partially denuded and cultured in SOF medium supplemented with 1.5% FCS, forskolin (10mM) and L-carnitine (1mM), following previously defined protocol (Lima, M.R. Thesis (Doctorate) - 74 p., 2015, http://hdl.handle.net/11449/136752) at 38.5°C in 5% CO2, 5% O2, 90% N2. FO was supplemented during IVM and/or at D3 (72h after fertilization) in the followings concentrations: 0mM (control), 10mM (treat.1) and 15 mM (treat.2). Experimental groups were: A: IVM(control)/D3(control); B: IVM(control)/D3(treat.1); C: IVM(control)/D3(treat.2); D: IVM(treat.1)/D3(control); E: IVM(treat.1)/D3(treat.1); F: IVM(treat.2)/D3(control); G: IVM(treat.2)/D3(treat.2). Five replicates were performed, totaling approximately 100 oocytes per group. Statistical analyses were performed in GraphPad Prism 9 software. Proportions were analyzed by Chi-Square Test ($\chi 2$). We detected that groups B (56/64 – 92,2%a) and F (85/95 – 89,5%a), despite being numerically higher in terms of cleavage rate, did not differ from the control group (58/71 – 81,7%ab). However, increased rates were detected for B and F groups in relation to other groups (C: 58/77 – 75,3%b; D: 81/107–75,7%b; E: 94/120 – 78,3%b; G: 63/116 – 54,3%c). Regarding blastocyst rates, B group (39/64 –60,9%a) presented similar rates in comparison to control (42/71 – 59,2%ab), with were higher than other groups (C: 34/77 – 44,2%bc; D: 45/107 – 42,1%c; E: 45/120 – 37,5%c; F: 36/95 – 37,9%c; G: 40/116 – 34,5%c). We concluded that the addition of 10mM forskolin in D3 does not harm embryonic development. Improvement of embryonic quality and cryosurvival is expected, which will be assessed in the following steps of this study.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Small extracellular vesicles from bovine follicular fluid isolated by distinct methods have different sizes and concentrations

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Resumo

Small extracellular vesicles (sEVs) are part of intercellular communication and can carry bioactive molecules like proteins and miRNAs. In reproductive research recent studies have demonstrate that bovine IVP can benefit from sEVs supplementation because they are present in different body fluids, such as follicular fluid (FF), and may mimic the physiologic conditions. However, the challenge is to define an accurate and reliable protocol for the sEVs isolation due to their high heterogeneity and overlapping biophysical properties. Thus, this study aims to characterize bovine FF sEVs isolated by three different methods through particle size and concentration using nanoparticle tracking analysis (NTA). For this, pairs of slaughterhouse ovaries were collected and characterized according to the stage of the estrous cycle. FF from ovaries were classified as stage 3 (Ireland et al. 1980. J Dairy Sci. 63:155–160) and the follicles between 3-6 mm in diameter were aspirated to collect its contents. The FF were grouped into pools, and each pool was formed by 4 to 6 single ovaries (n=7 pools). After FF was centrifuged and used for sEVs isolation by ultracentrifugation (UC), size exclusion chromatography (SEC; Izon Science qEV 35 nm; NZL) or precipitation (Exoquick-TC, System Biosciences; USA). All isolations were performed with 200 µL of FF and finally resuspended in 2 mL of 1×PBS Ca+2 Mg+2 free. In the UC, FF was filtered through a filter with a pore size of 0.22 µm and ultracentrifuged twice at 120000×g for 70 min at 4ºC. In SEC and precipitation methods, the FF were processed to isolate the sEVs following the manufacturer's instructions. To evaluate the concentration and size of particles we used the NTA (NS300 Build 3.1.45 Malvern). For that, 50 µL of the isolated sEVs were diluted in 950 µL 1×PBS Ca+2 Mg+2 free. For each sample, at least 3 videos of 30 seconds were made using the sCMOS camera, camera level 13, detection threshold 5 and controlled temperature at 38,5°C. The particle size and concentration data were submitted to the normality test (Shapiro-wilk) followed by ANOVA and Tukey's test, considering a significance level of 5%. The precipitation method (147.87 ± 2.49 nm) isolated FF sEVs similar to the UC (154.84 ± 2.69 nm) and smaller than the SEC (158.68 ± 2.89 nm) method (P=0.0172). The particles concentration was higher in FF isolated with the precipitation method (4.52x1011 ± 1.38x1011 particles/mL) followed by the SEC (3.95x1011 ± 1.61x1011 particles/mL) and UC (2.53x1011 ± 1.5x1010 particles/mL) methods (P<0.0001). Together, our results demonstrate that the isolation method recovers sEVs with different sizes and concentrations. These results suggest that distinct sEVs subpopulations are being separated depending on the method used which could impact the outcomes. Therefore, future analyses are needed to characterize the sEVs contents when different methods are used to separate them.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

DEVELOPMENTAL KINETICS OF BOVINE EMBRYOS EXPOSED TO ISOFLURANE

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Resumo

Isoflurane is an inhalation anesthesic widely used in veterinary and medical practice. The concentrations used on general anesthesia can change (0,3V% - 5V%), accordying to the species. During the process of IVF of mouses exposed to Isoflurane concentrations at 1,5%, similar to those used in anesthesia for ART, delayed the blastocyst development. The aim of this study was to investigate the effects of the anesthetic isoflurane on the kinetics of preimplantation embryo development. We used the bovine in vitro model of exposure during the third maternal-zygotic transition phase, on day 3. A total of 933 oocytes from slaughterhouse ovaries were selected. IVM was performed in TCM199 5% FCS medium at 38.5°C, 5% CO2. After 22-24h the oocytes were transferred to IVF plate with TALP medium. The drops were inseminated at 1x106 sperm/mL and placed for 22-24h at 38.5°C. After, zygotes were cultured in SOF medium at 5%CO2/90%N2/5%O2. On day 3, the groups were exposed to isoflurane forming the treatment groups: control group (CG) was not submitted to the anesthetic, and groups exposed to isoflurane for 1h, 3h, and 6h (G1h, G3h, and G6h, respectively). The exposure was performed in a modular incubator (Phorma, 2L) with maximum humidity, at 38.5°C, in an atmosphere with 5% CO2, 5% O2 balanced with N2. This chamber was connected to an anesthesia machine airflow rate of 6 L/min and vaporize concentrations of 3% isoflurane for 3 min (based on the method CHETKOWSKI, 1988). On day 7, the groups were evaluated by analysis of embryonic development stage (Mo; Bi; Bl; Bx) and embryonic kinetics, classifying them into slow (Mo + Bi) and fast (Bl + Bx) embryos. The test for homogeneity of proportions was used to compare the independent variables. The results found for blastocyst development stage at D7 indicated a significant difference between CG (Bi: 23.81%; Bl: 40.00%; Bx: 36.19%; p<0.001) and the others, as for the exposed groups G1h (Bi: 19.44%; Bl: 22.22%; Bx: 11.11%; p=0.739), G3h (Bi: 25.71%; Bl: 14.29%; Bx: 8.57%; p=0.209) and G6h (Bi: 26.92%; Bl: 19.23%; Bx: 0.00%; p=0.353) there was no statistical difference among them. Also on the seventh day, it was found that 50% of the embryos were in the morula stage (G1: 47.22%; G3: 51.43%; G6: 53.85%), while the CG (0%; p<0.001) had no embryos in this stage. In the evaluation of the kinetics of development there was a significant difference in the number of fast embryos in the CG compared to the other exposed groups, which were similar (CG: 76.19%; G1: 33.33%; G3: 22.86; G6: 19.23; p<0.001). Slow embryos predominated in all three exposure groups, differing from the control, which was significantly lower (CG: 23.81%; G1: 66.67%; G3: 77.14%; G6: 80.77%; p=0.856). These results indicate that there is an interference in the early developmental patterns of embryos exposed to isoflurane, interfering with developmental kinetics by delaying the embryonic events of compaction, blastocele opening and initial blastocyst formation.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Incidence of uterine disorders and gene expression of immunological markers in high- yielding Holstein cows supplemented or not with polyunsaturated fatty acids during peripartum period

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Resumo

The aim of this study was to investigate whether supplementation with polyunsaturated fatty acids (PUFAs), especially omega 3 (n-3), affected the incidence of uterine disorders as well as altered the gene expression of inflammatory cytokines during 28-35 days in milk (DIM) in high-yielding Holstein cows. A total of 36 animals were divided into two groups, as follows: group (1) control, those animals that received a supplement without PUFAs; and group (2) PUFA, those animals that received a supplement similar to group 1 + PUFA added. Supplementation of both groups was provided at the same times three times a day from 30 days before parturition until 35 days postpartum. To evaluate uterine disorders, the presence of fetal membrane retention, puerperal metritis and clinical endometritis (both evaluated by Metrickeck© and ustrasound) and subclinical endometritis (evaluated by endometrial cytology) were observed. To explore immune function, only those animals free of uterine affections or any disorder were used. For this, uterine swab samples were collected during 28-35 DIM for further analysis by performing a real-time polymerase chain reaction – qRT-PCR. For qualitative and unpaired variables Fisher's test was used at a significance level of 5%. For quantitative variables, data normality was initially evaluated using the Anderson Darling or Kolmogorov-Smirnov normality test at a significance level of 5%. To compare two unpaired variables, the unpaired T test was used for variables with normal distribution (parametric variables) or the Mann-Whitney test for variables with non-normal distribution (non-parametric variables). All tests were evaluated with a significance level of 5% ($P < 0.05$. No significant differences were observed between the control and PUFA groups regarding uterine conditions such as fetal membrane retention (0.0% vs.0.0%), puerperal metritis (0.0% vs. 5.9%), clinical endometritis (17.6% vs. 13.6%) and subclinical endometritis (3.5% vs.4.1%) respectively. Furthermore, the gene expression of IL-6, IL1-β and CCL5 was similar ($P > 0.05$) when comparing the control and PUFA group. Therefore, it was concluded that supplementation with PUFAs for high- yielding Holstein cows was not able to interfere in cases of retention of fetal membranes, metritis and endometritis, nor to affect the immune system through the gene expression of inflammatory cytokines such as IL -6, IL1-β and CCL5.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

MORPHOLOGICAL EVALUATION OF IN VITRO BOVINE EMBRYOS EXPOSED TO ISOFLURANE

Cínthia Medeiros Barriviera [1], GUILHERME BARIZÃO [1], Raiane Cristina Fratini de Castro [1], Giovanna Rafael Fernandes da Silva [1], Luiz Fernando Cassula Paiva [1], Dayane Aparecida dos Santos [1], Fábio Luiz Bim Cavalieri [1], Luiz Felipe Machado Velho [1], Isabele Picada Emanuelli [1]

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Resumo

Inhaled anesthesics are one of the most used drugs for inductions and in anesthesic maintenance. The concentration of Isoflurane used may change under different conditions. Similars concentrations used in anesthesia for asssited reproduction techniques (1,5%), were able to reduce blastocyst capacitations in mouse expossed during the in vitro fertilization process. The aim of this work was to evaluate the embryotoxic effects of isoflurane, in the embryonic phase of maternal-zygotic transition, on the morphological quality of bovine blastocysts produced in vitro. Oocytes were selected (grade 1 and 2) and matured in vitro in TCM199 5% SFB at 38.5°C at 5% CO2. After 22-24h, the oocytes were transferred to IVF plate with TALP medium and inseminated at a concentration of 1x106 sperm/mL, for 22-24 hours. After fertilization, zygotes were cultured in SOF medium, supplemented with fetal bovine serum and BSA, in 5%CO2/90N2/5%O2. On the third day of culture, the treatment groups were exposed to isoflurane for 1, 3, and 6 hours (G1h, G3h, and G6h, respectively); and the control group (CG) was not subjected to anesthesia. The exposure was in a modular incubator (Phorma, 2L), 38.5°C, in 5%CO2/90N2/5%O2. This chamber was connected to an anesthesia machine (Hipnos - RWR) adjusted to provide a total airflow rate of 6 L/min, and vaporize concentrations of 3% isoflurane (based on the method CHETKOWSKI, 1988). On day 7, the groups were evaluated for blastocyst rate and morphological quality according to IETS: Q1 - excellent or good with up to 15% extruded cells; Q2 - fair, between 15 and 50% extruded cells; Q3 - poor, more than 75% extruded cells; Q4 - incompatible embryonic development, less than 25% viable embryonic mass. On day 10 the hatching rate was evaluated. The test of homogeneity of proportions was used to compare the variables of blastocysts; hatching and embryonic qualities. The results obtained indicated that the blastocyst rate in CG (45.26%; p-value < 0.001) was higher when compared to the exposed groups G1h (8.05%), G3h (7.33%) and G6h (5.15%). Significantly higher rates of hatched embryos were obtained in CG (Be: 83.81%; p-value < 0.001), when compared to the other treated groups, while among the exposed groups there was no significant difference in hatching (42.11%, p=0.405; 29.41%, p=0.196; 8.33% p=0.102, respectively G1, G3, G6). In the evaluation of embryo quality, there was a significant difference (p-value < 0.001), comparing the CG (Q1: 48.57%; Q2: 37.14%) with the other exposed groups G1h (Q1: 31.58%; Q2: 36.84%) G3h (Q1: 17.65%; Q2: 35.29%) G6h (Q1: 8.33%; Q2: 8.33%), observing superior embryo quality in the CG. Based on the results obtained, we conclude that the groups that were exposed to isoflurane obtained embryos with inferior morphological quality time-dependently. Exposure to isoflurane in the embryonic genome activation stages directly interfered with blastocyst production, hatchability and the quality of the resulting blastocysts.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Transcriptomic comparison between bovine sires with differing field fertility.

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Resumo

The objective of this study was to determine the differentially abundant genes between sires of known fertility, both in the conceptus and the uterus. The sires used have previously been reported to have drastically different levels of field fertility; however, they had passed all normal standards for frozen thawed semen. Bos indicus beef heifers (n=45) were subjected to estrous synchronization and embryo transfer on day 7 with IVP embryos from one of two sires (High Fertility and Low Fertility Sire) and pregnancies were later confirmed at slaughter. Samples were collected from the trophectoderm and uterus on day 25 and 36 of gestation to attain samples for RNA sequencing. Total RNA was isolated from tissue samples using the RNeasy kit (QIAGEN; Hilden, Germany) per manufacturer's instructions. The RNA sequencing was conducted using an Illumina platform. Sequences were aligned to the reference genome ARS-UCD 1.2. Differentially expressed genes (DEGs) between sires and by tissue were determined using edge-R package from R. The false discovery rate used was 0.05. On day 25 15,755 genes were identified in the trophectoderm between the two sires. Of those, 11 genes were downregulated in the Low Fertility Sire compared to the High Fertility Sire and an additional 6 genes were upregulated in the high fertility sire. Additionally, 16,044 genes were identified within the caruncle where the Low Fertility Sire resulted in 2 downregulated genes and the High Fertility Sire resulted in no upregulated genes. On day 36 17,080 genes were observed in the trophectoderm sample where the Low fertility Sire resulted in 23 downregulated genes in comparison with the High Fertility Sire that resulted in 4 upregulated genes, furthermore 17,843 genes were observed in the caruncle sample resulting in 8 downregulated genes for the Low Fertility Sire whereas 21 genes were upregulated for the High Fertility Sire. Gene ontology analysis reported differentially expressed genes in the High Fertility Sire compared to Low Fertility Sire were associated with hematology, immunology and reproduction. Of particular interest was the transferrin gene (TF) that is known to be responsible for the transport of iron from sites of absorption and heme degradation to those of storage and utilization, but it is also known for its role in stimulating cell proliferation. Additionally, the spermatogenesis associated 22 gene responsible for gamete generation, homologous chromosome pairing at meiosis, meiotic DNA repair synthesis was also found downregulated in between sires. These data suggests that other underlying factors are at work regarding conception rate and current methods are insufficient for sire selection and fertility testing. In conclusion, different paternal genome results in differences at both trophectoderm and uterus level in bos indicus beef cows. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2019-67015-28998 from USDA NIFA.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Influence of sub-clinical endometritis on early pregnancy predictors and pro-inflammatory cytokines in circulating immune cells in dairy cows

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Resumo

We aimed to evaluate the influence of subclinical endometritis (SCE) and pregnancy status on expression of genes stimulated by interferon (ISGs) and inflammation in circulating immune cells in dairy cows. In Experiment 1, peripheral blood mononuclear cells (PBMCs) were isolated from cows considered healthy or with SCE (n=6/group) on Days 0 (estrus) and 7 (diestrus) of a sinchronized estrous cycle. In Experiment 2, on day 21th after a timed-artificial insemination, cows were evaluated by ultrasonography to assess luteal blood perfusion and PBMCs were isolated. Thirty-two days after insemination, cows were classified into: healthy pregnant (n=7), pregnant with SCE (n=4), healthy non-pregnant (n=8), and non-pregnant with SCE (n=10). SCE diagnose was performed by the Cytrobush technique. For classification of SCE occurrence, only cows with ≥ 5.5% of PMN and ≥ 5.0% of PMN were considered with SCE, in Experiment 1 and 2, respectively. Were considered cows without any uterine disease (NUD group), in Experiment 1, ≤ 3.0% of PMN, and , in Experiment 2, cows with ≤ 2.0% of PMN. Gene expression of ISGs (ISG15, OAS1, MX1 and IFI6) and pro-inflammatory cytokines (IL1-β, TNF-α and IFN-γ) were determined. Expression of ISG15, MX1, IFI6, TNF-α and IFN-γ did not differ (P>0.1) between SCE and healthy cows and between Days 0 and 7. However, a greater (P=0.02) expression of OAS1 (1.4-fold) and IL1-β (19.3-fold) in PBMCs was observed on Day 7 than Day 0. In Exp.2, ISG15 abundance was 2.5-fold greater (P=0.0008), TNF-α was 2.2-fold greater (P=0.05), and IL1-β tended (P=0.06) to be 2.4-fold greater in pregnant than non-pregnant cows. Luteal blood perfusion was greater (P=0.01) in pregnant animals. In conclusion, OAS1 and IL1-β are transcripts upregulated in PBMCs at diestrus, regardless of SCE occurrence. Pro-inflammatory cytokines are not affected by SCE occurrence, but IL1-β and TNF-α are upregulated in pregnant animals on day 21th after insemination. ISG15 abundance is a good pregnancy predictor, regardless SCE presence.